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Attorney Docket No. 9056-5CT PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Federico Mailland
Serial No.: 10/016,005
Filed: November 1, 2001
For: METHODS OF MAKING SUSTAINED-RELEASE PHARMACEUTICAL COMPOSITIONS OF ERGOT ALKALOIDS HAVING IMPROVED BIOAVAILABILITY AND COMPOSITIONS THEREOF

Bet 4-26-02

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR §1.132

I, Federico Mailland, do hereby declare and say as follows:

1. I am the sole inventor on the above-referenced patent application and am familiar with the contents thereof. I have reviewed the final Official Action mailed August 1, 2001 and am familiar with the contents thereof. I have also reviewed U.S. Patent No. 4,737,499 to Giger and U.S. Patent No. 5,069,911 to Züger, which are cited in the final Office Action.

2. I received a degree in Medicine from the University of Milan School of Medicine in 1975. I received a post-graduate diploma in Pharmacology from the University of Milan School of Medicine in 1977. I received a post-graduate diploma in Dietology from the University of Milan School of Medicine in 1980. From 1971 to 1974, I was employed by Società Italiana Schoum S.p.A. as a medical reporter. From 1974 to 1977, I was employed by Pierrel S.p.A. as Assistant to the Medical Director where my duties included research in developing nutritional products, antibiotics and local anesthetic agents. By the end of 1977, I became the Medical Director of Pierrel S.p.A. where my clinical research interests pertained to antibiotics and parenteral nutrition. I was Assistant to the General Manager of Poli Industria Chimica from 1981 to 1982, and then became the Director of Research and Development until 1991. During this time, I directed research in the areas of neuroscience and immunology and also developed products in cardiovascular and respiratory areas. I also directed pre-clinical development and phase 1-4 clinical trials throughout Europe. In 1992, I became the Scientific Director of Poli Industria Chimica S.p.A. and was responsible for national and international regulatory affairs and was also instrumental in filing numerous

patents worldwide. In 1997, I became the Scientific Director of Poli Management S.p.A. where my responsibilities included managing research and development activities, mainly in the areas of dermatology and neuroscience, and managing the regulatory affairs for all Companies of the group. During this time, I also instituted a Pharmacovigilance Unit. Since April 2001, after the headquarters of Poli Group was transferred to Polichem S.A. in Lugano, Switzerland, I have been employed as the Scientific Director of Poli Group in Lugano where my responsibilities have included managing research and development activities, Pharmacovigilance and managing worldwide regulatory affairs. I have authored approximately 90 papers and 100 other communications. As a responsibility of my current employment, I am involved in improving the bioavailability of ergot derivatives through administration using a sustained-release delivery system.

3. The investigations described in Sections 4-6 below were carried out at two locations. Technical investigations were conducted at Poli Industria Chimica S.p.A. in Milan, Italy under my direction and supervision, and clinical testing was conducted at Polichem S.A. in Lugano, Switzerland under my direction and supervision. These studies provide bioavailability comparison data demonstrating the improved bioavailability of our sustained-release formulation compared to that of a conventional tablet. Additionally, these studies demonstrate the improved bioavailability of our sustained-release formulation compared to sustained-release formulations prepared according to the Züger reference.

4. Methods and results of in vitro dissolution tests conducted to confirm the sustained release for a compound of the present invention and compounds prepared according to Züger are presented at Tab 1. Table 1 at Tab 1 shows the percent release of α-dihydroergocryptine of a conventional tablet formulation. The percent release of α-dihydroergocryptine of sustained release α-dihydroergocryptine prepared according to the formulation of the present invention is shown at Table 2 at Tab 1. Percent release of α-dihydroergocryptine from a sustained release capsule (designated "109/7") prepared according to a formulation disclosed by Züger is shown at Table 3 at Tab 1. Table 4 at Tab 1 shows the percent release of α-dihydroergocryptine from another sustained release capsule (designated "109/8") prepared according to a formulation disclosed by Züger. These results

show that the sustained release compounds prepared according to the formulations of the present invention or the formulations as disclosed by Züger, demonstrate sustained release capability.

5. Methods and results of clinical comparison tests to evaluate the pharmacokinetic profile and bioavailability of α-dihydroergocryptine in oral sustained release tablets prepared according to the formulation of the present invention and prepared according to the formulation of Züger are shown at Tab 2. More specifically, the objective of this study was to evaluate pharmacokinetic characteristics and the bioavailability of *α*-dihydroergocriptine in oral sustained-release tablets according to the present invention as described at Tab 1 (hereafter referred to as "B") in comparison to the following: a conventional tablet according to the conventional tablet formulation as previously described at Tab 1 (hereafter referred to as "A"), a sustained-release capsule prepared according to the method disclosed by Züger (US 5,069,911) as previously designated at Tab 1 as 109/7 (hereafter referred to as "C"), and a sustained-release capsule prepared according to the formulation disclosed by Züger (US 5,069,911) previously designated at Tab 1 as 109/8 (hereafter referred to as "D").

The study was divided into two phases. During the first phase, the pharmacokinetics of "A" and "B" were compared in an open label, crossover, 2 period design. Twelve male volunteers were randomly assigned to the two treatment sequences, separated by a one-week washout period. The drug was administrated orally in the morning under fasted conditions in a single dosage of 20 mg. Blood samples were obtained by an indwelling cannula at specific time points up to 72 hours after administration of the drug.

6. The data at Tables 1 and 2 at Tab 2 show that all the sustained release formulations significantly reduce the peak concentration (C_{max}) and delay the time to peak concentration (T_{max}). The data correspond to a slower absorption rate and a dramatic reduction of the burst that usually occurs after drug administration using a conventional formulation. Moreover, the data at Table 2 at Tab 2 show that the bioavailability measured by AUC of the sustained release formulation "C" and "D" prepared according to the method disclosed by Züger (US 5,069,911), is similar or slightly lower than the conventional tablet

"A." On the contrary, the bioavailability of sustained release formulations of the present invention, as measured by AUC, is surprisingly higher than the bioavailability obtained with the conventional tablet.

7. From these results, it is clear that the ergot derivative sustained-release formulation of the present invention increases the bioavailability of the administered drug as compared to conventional delivery systems and as compared to the formulations disclosed by Züger.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Federico Mailland

Lugano, 20th March 2002
Date

IN VITRO DISSOLUTION TESTS

A. Composition of conventional tablet formulation

α-Dihydroergocryptine Mesylate	20.0 mg
lactose	148.0 mg
microcrystalline cellulose	70.0 mg
croscarmellose	6.0 mg
magnesium stearate	4.0 mg
polyvinylpyrrolidone	2.0 mg.

B. Composition of sustained- release tablets of the present invention

α-Dihydroergocryptine Mesylate	20.0 mg	4.0%
[1]Cellactose®	368.6 mg	73.72%
[2]Methocel K4M®	44.0 mg	8.8%
[3]Methocel K15M®	19.4 mg	3.88%
[4]Carboxymethyl cellulose Sodium 7-HXF	4.0 mg	0.8%
Magnesium stearate Ph. Eur.	4.0 mg	0.8%
Talc Ph. Eur.	40.0 mg	8.0%

[1]Composed of 75% lactose and 25% microcrystalline cellulose, commercially available from Meggle GmbH of Wasserburg, Germany.
[2]Hydroxypropylmethylcellulose USP type 2208; 4,000 mPa·s, commercially available from Colorcon of West Point, Pennsylvania.
[3]Hydroxypropylmethylcellulose USP type 2208; 15,000 mPa·s, commercially available from Colorcon of West Point, Pennsylvania.
[4]Medium viscosity grade.

Experimental method

The formulation was prepared by mixing in a cube mixer (300 liter) for 15 minutes, 73.72% of Cellactose as a direct compressible agent, 8.8% of Methocel K4M and 3.88% of Methocel K15M and 0.8% Carboxymethyl cellulose Sodium as swellable controlled-release agents, 4% α-Dihydroergocryptine Mesylate as the active ingredient and 8% Talc as filler. After adding 0.8% of Magnesium Stearate, the blend was mixed further for 15 minutes. The

blend is then pressed on a rotary tabletting machine (Ronchi 23N) equipped with capsular punctions Ø 12 mm.

Tablet testing

Standard pharmaceutical test methods and equipment were used to determine the following:

Hardness: 7.5 Kp (Schleuniger 4M)

Friability: 0.13%

Mean weight: 500 mg

Thickness: 5 mm

C. Composition of sustained release tablets prepared according to Züger (109/7)

α-Dihydroergocryptine Mesylate	20.0 mg	5.0%
Lactose FU	176.0 mg	44.0%
[1]Aerosil 200	2.0 mg	0.5%
[2]Precirol AT05 (glycerol distearate)	20.0 mg	5.0%
[3]Methocel E4M Premium	180.0 mg	45.0%
Magnesium stearate	2.0 mg	0.5%

[1]Composed of Silicon Dioxyde, commercially available from Giusto Favarelli SpA, Italy.
[2]Glycerol distearate, commercially available from Giusto Favarelli SpA, Italy.
[3]Hydroxypropylmethylcellulose USP type 2208, 4.000 mPa·s, commercially available from Colorcon of West Point, Pennsylvania.

Experimental method

A pre-mixing composed of 5% α-Dihydroergocryptine Mesylate as the active ingredient, 44% Lactose and 0.5% of Aerosil as fillers, was prepared through a sieve. To this blend, 45% of Methocel E4M Premium and 5% Precitol AT05 were added and mixed for 10 minutes. After adding 0.5% of Magnesium Stearate, the blend was mixed further for 15 minutes. The blend was then encapsulated using a manual encapsulation machine.

D. Composition of sustained release tablets prepared according to Züger (109/8)

α-Dihydroergocryptine Mesylate	20.0 mg	5.0%
Lactose FU	56.0 mg	14.0%
[1]Aerosil 200	2.0 mg	0.5%
[2]Precirol AT05 (glycerol distearate)	200.0 mg	50.0%
[3]Methocel E4M Premium	120.0 mg	30.0%
Magnesium stearate	2.0 mg	0.5%

[1]Composed of silicon Dioxyde, commercially available from Giusto Favarelli SpA, Italy.
[2]Glycerol distearate, commercially available from Giusto Favarelli SpA, Italy.
[3]Hydroxypropylmethylcellulose USP type 2208, 4.000 mPa·s, commercially available from Colorcon of West Point, Pennsylvania.

Experimental method

A pre-mixed composed of 5% α-Dihydroergocryptine Mesylate as the active ingredient, 14% Lactose and 0.5% of Aerosil as fillers, was prepared through a sieve. To this blend, 30% of Methocel E4M Premium and 50% Precirol AT05 were added and mixed for 10 minutes. After adding 0.5% of Magnesium Stearate, the blend was mixed further for 15 minutes. The blend was then encapsulated using a manual machine.

E. In vitro dissolution test results

Dissolution test were conducted according to USP XXIII, p. 1792, Apparatus 2, 500 ml 0.01 N HCl, 50 rotations / min.

Table 1. Conventional Tablet Formulation

Time (hours)	% Release of α-dihydroergocryptine
After 0.5	96.52%

Table 2. Sustained Release of α-dihydroergocryptine of the Present Invention

Time (hours)	% Release of α-dihydroergocryptine
after 1	22.6%
after 4	48.0%
after 8	80.2%

Table 3. Sustained Release of α-dihydroergocryptine of the Züger (109/7)

Time (hours)	% Release of α-Dihydroergocryptine
1	20.2 %
2	31.8 %
4	48.1 %
6	58.0 %
8	68.6 %
20	94.2 %

Table 4. Sustained Release of α-dihydroergocryptine of the Züger (109/8)

Time (hours)	% Release of *a*-Dihydroergocriptine
1	14.6 %
2	25.5 %
4	39.3 %
6	48.6 %
8	59.1 %
20	88.4 %

TAB 2

CLINICAL COMPARISON TESTS

Objective

The objective of this study was to evaluate pharmacokinetic characteristics and the bioavailability of α-dihydroergocriptine in oral sustained-release tablets according to the present invention as described at Tab1 (hereafter referred to as "B") in comparison to the following: a conventional tablet according to the conventional tablet formulation as previously described at Tab1 (hereafter referred to as "A"), a sustained-release capsule prepared according to the method disclosed by Züger (US 5,069,911) as previously designated at Tab 1 as 109/7 (hereafter referred to as "C"), and a sustained-release capsule prepared according to the formulation disclosed by Züger (US 5,069,911) previously designated at Tab 1 as 109/8 (hereafter referred to as "D").

The study was divided into two phases. During the first phase, the pharmacokinetics of "A" and "B" were compared in an open label, crossover, 2 period design. Twelve male volunteers were randomly assigned to the two treatment sequences, separated by a one-week wash-out period. The drug was administrated orally in the morning under fasted conditions in a single dosage of 20 mg. Blood samples were obtained by an indwelling cannula at specific time points up to 72 hours after administration of the drug.

The plasma concentrations throughout the observation period are depicted in Figure 1 (attached). The results of the pharmacokinetic analysis carried out on the plasma concentrations are reported in Table 1 (expressed as mean values).

Table 1. Pharmacokinetic analysis of conventional release formulation and sustained-release formulation of the present invention

	"A" Conventional Release Formulation	**"B"** Sustained-Release Formulation of Present Invention
C_{max} (ng/ml)	397.0	153.4
T_{max} (h)	0.8	6.9
$AUC_{0\rightarrow t}$ (ng h/ml)	576.2	2006.0

In the second phase, the pharmacokinetics of "A", "C" and "D" were compared in an open label, crossover, 3 period design. Six male volunteers were randomly assigned to the three treatment sequences, separated by a one-week wash-out period. The drug was administered orally in the morning under fasted conditions in a single dosage of 20 mg. Blood samples were obtained by an indwelling cannula at specific time points up to 722 hours after administration of the drug.

The plasma concentrations throughout the observation period are depicted in Figure 2 (attached). The results of the pharmacokinetic analysis carried out on the plasma concentrations are reported in Table 2 (expressed as mean values).

Table 2. Pharmacokinetic analysis of conventional release formulation and sustained-release formulations according to Züger.

	"A" Conventional Release Formulation	"C" Sustained-Release Formulation 109/7	"D" Substained-Release Formulation 109/8
C_{max} (ng/ml)	309.6	38.2	39.0
T_{max} (h)	0.6	7.3	6.7
$AUC_{0\rightarrow t}$(ng h/ml)	727.1	552.4	418.8

These data clearly show that all the sustained-release formulations, namely "B", "C" and "D, " significantly reduce and delay the peak concentration. These data correspond to a slow absorption rate and a dramatic reduction of the burst that usually occurs after administration of a conventional formulation.

The bioavailability measured by AUC of the sustained release formulation "C" and "D" prepared according to the method disclosed by Züger (US 5,069,911), is similar or slightly lower than the conventional tablet "A." On the contrary, the bioavailability of sustained release formulations of the present invention, as measured by AUC, is surprisingly higher than the bioavailability obtained with the conventional tablet.